A simple and low cost DNA amplifier

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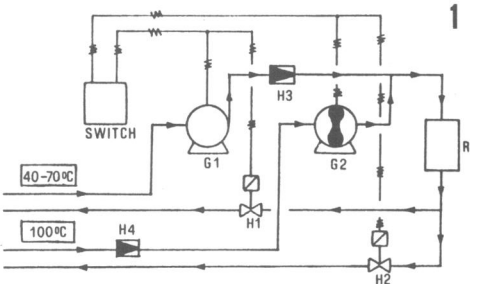
We describe here a simple and low cost laboratory instrument which carries out an automatic amplification of the DNA by means of the polymerase chain reaction, PCR (Saiki et al. 1985) using the Thermus aquaticus DNA polymerase. The DNA amplifier works by generating thermic square waves of appropriate amplitude and frequency inside a small reaction chamber where the micro-tubes which contain the PCR reagents are kept. The system takes advantage of the fact that virtually every molecular biology laboratory is equipped with one or more thermostatic water bath.

The thermic square waves are produced in the following way (Fig.1): warm (40-70 °C) water from a water bath is pumped through the reaction chamber (R) and returns to the bath through the solenoid valve (H1) which is on when the pump (G1) is in action. During this time the pump (G2) and the solenoid valve (H2) are kept out. After a certain time (6 min in our experiments) the electronic switch stops pump G1, turns off the solenoid valve H1 and turns on both pump G2 and the solenoid valve H2. Hot water is sucked from the boiling pot and pumped through the reaction chamber for ≥ 1 min and then returned to the pot. Then the cycle is repeated. The effect of the short high temperature phase of the cycle is to denature the DNA. The annealing of the oligonucleotide primers and their elongation takes place during the longer low temperature phase.

Technical comments

G1 is a domestic motor-driven 150 W centrifuge pump; G2 is a 130 W (2 l/min flow) laboratory peristaltic pump, we chose this type of pump as it insures good thermic insulation. H1 and H2 are 9 W solenoid valves. The reaction chamber (R) is made of 1 cm thick perspex , its external dimensions without the lid are 10x10x3.5 cm; the chamber lid is held in place by four screws and wing nuts and is made watertight by a rubber gasket; the reaction chamber inlet and outlet are made of brass tubing; they are fitted in a staggered position on opposite sides of the perspex box; a micro-tube rack converted from a plastic rack for 1000 µl tips is fitted into the reaction chamber; the rack can contain up to thirty-five 0.5 ml micro-tubes. The valves H3 and H4 prevent high temperature water from flowing into the low temperature water bath and vice versa ; the valve H3 is a small nylon valve for laboratory purposes, it has the additional function of reducing the flow of pump G1; H4 is a bronze flap valve for domestic heating plants, this valve is fitted at the end of the hot water



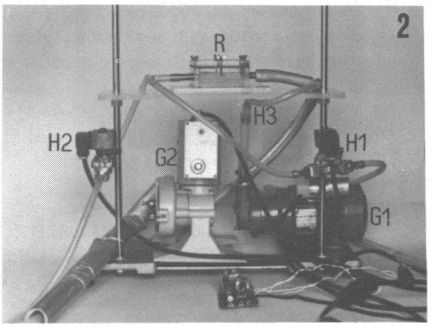
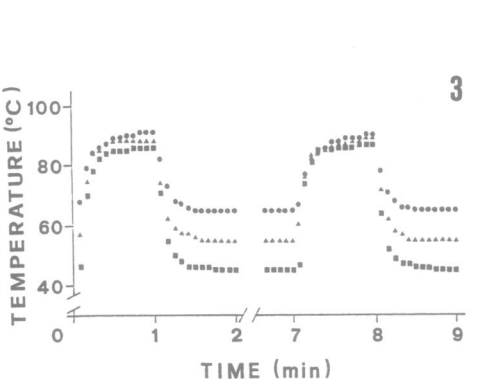


Fig. 1 Diagram of the DNA amplifier

Fig. 2 A view of the DNA amplifier assembled in the author's laboratory. The switching unit can be observed in the foreground. The valve H4 does not appear in this picture.



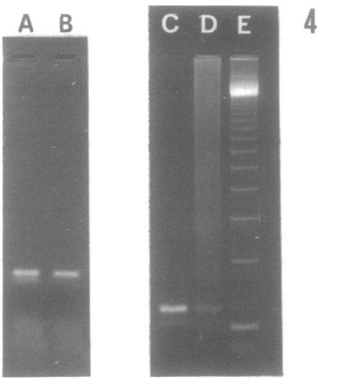


Fig. 3 Profiles of the thermic square waves of different amplitude which can be produced by the DNA amplifier. In this case the switch was set at 1 and 6 min respectively. The measures were obtained by inserting a thermocouple in the reaction chamber outlet. The symbols ■, ▲, and ● indicate that the water bath was set at 45, 55 and 65 °C respectively.

Fig. 4 Test run of the DNA amplifier: A) three-step manual amplification (20 cycles) of a 102 bp genomic sequence of fungal DNA (Rollo et al. 1987); B) two-step fully automatic amplification (20 cycles) of the same DNA by the DNA amplifier described in the text. C, D) two-step fully automatic amplification (30 cycles) of the followingDNA sequences: C) nucleotide sequence from -180 to -30 of the mitochondrial cytochrome c oxidase subunit I gene from fertile maize (Isaac et al. 1985); D) a 150 bp portion of the H2a clone from a family of highly repeated maize sequences (Viotti et al. 1985). E) size marker (BRL "123 bp DNA ladder").
In (A), DNA denaturation, annealing and elongation were performed at 91, 37 and 45 °C and for 1', 1' and 6' respectively. In (B, C, D), DNA denaturation was performed at about 87 °C for 1.4', and annealing+elongation at 45 °C for 6'. In all the cases 1 unit Thermus aquaticus DNA polymerase was added to the sample at the first amplification cycle. The remaining experimental conditions were as indicated by the manufacturer of the polymerase (Biolabs). The amplification products were fractionated on 2.5 % agarose.

suction pipe and is kept submerged in the boiling water. Both the hot and warm water flow through 8 mm (inner diameter) silicon tubes. In order to prevent heat losses the hot water tubings are externally insulated. The switching unit consists of a NE555 timer chip which drives a relay, the switching times can be modified to a certain extant by two potentiometers.
The whole system works well(see Fig. 3 and 4) and it is now routinely used in our laboratory
Any further information concerning the amplifier and its use can be obtained from the authors.

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